On and Off: A Dual Role for Cysteine Protease Autoprocessing of C difficile Toxin B on Cytotoxicity vs Proinflammatory Toxin Actions?

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EDITORIAL

On and Off: A Dual Role for Cysteine Protease Autoprocessing of *C difficile* Toxin B on Cytotoxicity vs Proinflammatory Toxin Actions?

Clostridium difficile is the leading bacterial cause of health care–associated diarrhea in the developed world. In recent years, there has been an alarming increase in the incidence and severity of *Clostridium difficile* infections (CDIs), leading to increased academic, public health, and drug development research efforts on this bacterium. Two major virulence factors of *C difficile* are its toxin A (TcdA) and toxin B (TcdB). Understanding the mechanisms of action of these toxins is important to advance knowledge of CDI pathogenesis and identify new targets for prevention and therapy.

TcdB, similar to TcdA, is a large single-chain protein that contains at least 4 distinct domains: the N-terminal glucosyltransferase enzymatic domain (GTD), cysteine protease enzyme domain (CPD), a putative translocation domain, and a C-terminal receptor binding domain. After receptor-mediated endocytosis and/or alternative entry pathways, the translocation domain mediates translocation of the CPD and GTD enzymatic regions into the cytosol where CPD self-cleaves in the presence of Inositol hexakisphosphate and releases GTD from the rest of the toxin.[1] GTD inactivates Rho guanosine triphosphatases, which are key cell signaling molecules, leading to cytotoxicity. Thus, CPD plays a key role in the cytosolic delivery of GTD to turn on cytotoxicity.

The proinflammatory activities of TcdB in human colon tissues and monocytes are well documented. Inactivation of RhoA results in the stimulation of the pyrin/apoptosis-associated speck-like protein containing a caspase recruitment domain inflammasome,[2] which is one of the main signaling pathways used by these toxins to trigger the inflammatory response. However, the relationship between toxin's cytotoxicity and proinflammatory activity has remained elusive. Although GTD-deficient toxins failed to induce acute intestinal responses in a number of studies,[3,4] indicating an essential role of GTD, there also have been reports suggesting that GTD is not required for the induced proinflammatory response of TcdB.[5]

In a new study in the current issue, Zhang et al[6] made the novel and exciting observation that the CPD autoprocessing activity of TcdB acts as an off switch for proinflammatory activity and an on switch for cytotoxicity. By analyzing inflammatory responses in mouse ilea loops, human tissues, and immune cells, they found that blocking autoprocessing of TcdB by mutagenesis or chemical inhibition resulted in reduced cytotoxicity of the toxin, but surprisingly enhanced its proinflammatory activities in a ligated mouse ileal loop model. Zhang et al[6] further validated in ex vivo human colonic tissues and immune cells that a noncleavable mutant TcdB was significantly more potent than the wild-type toxin in the induction of proinflammatory cytokines.

This study suggests a dual role of CPD-mediated autoprocessing, which regulates the relative outcomes of cytotoxicity and proinflammatory induction. There have been suggestions that CPD inhibition could trap TcdB in the endosome, thereby mitigating cytotoxic effects. However, this study suggests that there may be an unintended consequence to CPD inhibition in the form of increased inflammatory pathway activation. The question remains, however, which of the 2 components, cytotoxicity or proinflammatory responses, contribute more to disease pathogenesis.

The investigators showed that autocatalytic processing-deficient TcdA or TcdB mutants still have some cytotoxic activity, but at a reduced level compared with wild-type toxins. However, the proinflammatory activity was enhanced significantly. This may have alarming implications for drug development using CPD as targets. This study did not use infectious murine models of CDI, but it should be noted that in vivo studies in such models have found that CPD inhibitors reduced the overall severity of CDI.[7,8] Moreover, TcdB from hypervirulent *C difficile* shows increased autoprocessing efficiency.[9]

Although this study opens up many questions, it provides a new component of *C difficile* toxin mechanism of action, particularly in understanding how endosomal tethering, GTD domain localization, and/or release impact the inflammatory response. As such, it exposes a new and fascinating aspect to our understanding of the virulence of this difficult bacterium.

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Conflicts of interest
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